EXHIBIT 1

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Local Production of Complement Proteins in Rheumatoid Arthritis Synovium

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Objective. **Complement has been repeatedly implicated in the pathogenesis of rheumatoid arthritis (RA) based on studies showing reduced levels of native complement components and increased levels of complement metabolites in plasma, synovial fluid (SF), and synovial tissue (ST) of RA patients. However, there is limited information on local production and activation of key factors of the complement cascade in RA synovium and their potential modulation by novel anticytokine therapies. This study was undertaken to characterize the expression of complement proteins and receptors in RA SF and ST.**

Methods. **Using in situ hybridization, immunohistochemistry, and Western blot techniques, we assessed the presence of complement proteins C3, factor B (FB), and C5b–9, as well as the expression of complement receptors C3aR and C5aR in rheumatoid synovium. C3 and FB levels in SF were determined by enzyme-linked immunosorbent assay. Functional assessment was performed by examining the effects of soluble tumor necrosis factor receptor (sTNFR) p55 gene transfer in the SCID mouse model of RA.**

Results. **Complement proteins and receptors could be localized in all RA synovial specimens, whereas in osteoarthritis (OA) synovium, only a few, single cells expressed complement proteins and receptors. No differences were noted in the concentration of C3 between RA and OA in SF; however, FB levels were markedly reduced in RA versus OA SF. In RA synovium, in contrast to OA synovium, local expression of complement factor and complement receptor messenger RNA was found throughout the various ST compartments, suggesting that activation of the complement cascade occurs in all parts of the rheumatoid synovium. Moreover, C5aR expression was up-regulated following overexpression of sTNFR p55 by adenovirus-based gene transfer.**

Conclusion. **In summary, local complement production and activation may play an important role in RA, and specific modulation and inhibition of local complement production could be an attractive therapeutic target for RA.**

Rheumatoid arthritis (RA) is a severe chronic disease characterized by inflammation of synovial tissue (ST) in joints, tendon sheaths, and bursae, which causes pain and dysfunction and ultimately leads to destruction of these structures. To date, the pathogenesis of RA is not fully understood, and treatment options are still limited to symptomatic and nonspecific immunosuppressive therapies. RA is often regarded as a predominantly T cell–related disorder (1,2), and, as in other T cell–driven processes (e.g., experimental allergic encephalitis [3]), there is increasing evidence for an important role of components of the complement cascade in the pathophysiology of RA. In general, the complement cascade is involved in the induction and progression of inflammation reactions and is a major defense system against various pathogenic agents, including bacteria, viruses, and other antigens (4–6). Inappropriate activation, how-

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Table 1. Clinical data on the patients*

Patient	Age/sex	ESR, mm/hour	WBCs/nl	Medication
RA06	73/F	37	7.8	MTX, Pred.
RA08	55/F	45	8.7	–
RA09	72/M	29	14.5	–
RA11	76/F	15	9.0	GST, Pred.
RA55	67/F	32	11.3	Pred.
RA56	73/F	35	7.0	Pred., HCQ
RA57	70/F	10	7.2	Pred.
RA58	68/M	6	6.7	HCQ
RA63	76/F	32	8.7	Pred.
RA66	62/F	10	14.3	Pred., SSZ
RA67	60/F	103	6.3	HCQ
OA03	77/F	9	10.4	–
OA44	74/M	7	9.0	–
OA45	72/M	7	5.1	–
OA46	74/F	24	8.9	–
OA47	74/F	25	7.4	–

* ESR = erythrocyte sedimentation rate; WBCs = white blood cells; RA = rheumatoid arthritis; MTX = methotrexate; Pred. = prednisone (oral); GST = gold sodium thiomalate; HCQ = hydroxychloroquine; SSZ = sulfasalazine; OA = osteoarthritis.

ever, can lead to tissue damage and manifestation of disease (4–7).

Reduced levels of native complement components and increased levels of complement metabolites in plasma, synovial fluid (SF), and ST of RA patients have implicated complement in the pathogenesis of RA (8–19). The complement components found in SF and ST in RA are thought to be derived from serum, synthesized by synovial mononuclear phagocytes (20), and/or the ST itself (8,16,17); however, this remains controversial. In this study, we demonstrated that complement components and receptors are readily produced in situ in the inflamed RA synovium. Using in situ hybridization, immunohistochemistry, and Western blot techniques, we were able to localize the sites of complement messenger RNA (mRNA) expression in rheumatoid synovium. On the basis of these results, the potential role of complement activation in RA is discussed.

PATIENTS AND METHODS

Tissue specimens. ST and SF samples were obtained from 5 patients with osteoarthritis (OA) and from 12 patients with RA that met the revised RA criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (21). The tissue and SF samples were provided by Professor Wessinghage (University of Regensburg), according to the regulations of the local ethics committee. Immediately after orthopedic arthroscopy or surgery, the tissue samples and the SF were snap-frozen in OCT TissueTek embedding medium (Miles, Elkhart, IN) and stored at −70°C. Table 1 shows the clinical data on the patients.

Preparation of riboprobes. Human C3 complementary DNA (cDNA) plasmid was linearized using *Bgl* II or *Xho* I endonucleases (Promega, Madison, WI) for generation of antisense and sense riboprobes, respectively. Human C3a receptor (C3aR) cDNA plasmid was linearized using *Xho* I or *Bgl* II endonucleases for generation of antisense and sense riboprobes, respectively. C5aR riboprobes were prepared by linearizing a human C5aR cDNA clone, using *Xba* I or *Cla* I endonucleases (Promega) for generation of antisense and sense riboprobes, respectively. In vitro transcription was performed using an RNA transcription kit (Promega) and T7 or SP6 RNA polymerases (Promega). Preparation of factor B (FB) riboprobes was performed accordingly. All experiments were performed according to previously described protocols (22–25).

In situ hybridization. In situ hybridization using digoxigenin-labeled riboprobes was performed on 8-μm-thick RA or OA samples, as previously described (24). Briefly, sections were fixed for 1 hour in 3% paraformaldehyde (pH 7.4) and hybridized for 16 hours at 50°C with riboprobes diluted 1:10 in hybridization buffer (Fisher Scientific, Fair Lawn, NJ). After hybridization, 3 consecutive washes at increasing stringency (final wash in 0.1× saline–sodium citrate containing 0.1% sodium dodecyl sulfate [SDS] for 5 minutes) were performed at 50°C. Immunologic detection was performed by incubating slides with anti-digoxigenin alkaline phosphatase–conjugated Fab (Boehringer Mannheim, Indianapolis, IN), followed by a BCIP/nitroblue tetrazolium color substrate solution (Sigma, St. Louis, MO).

Immunologic detection. For detection of C3aR, C5aR, and CD45RO, sections were analyzed using the immunogold-silver technique described by Roth et al (26). Briefly, sections were fixed in acetone and then blocked in 2% normal horse serum to block nonspecific binding. Primary antibodies were diluted in 1% normal goat serum/2% milk/0.05*M* Tris buffer (pH 7.4) at 12 μg/ml (for tissue) and 60 μg/ml (for cells). After immunohistochemical staining, sections were rinsed and fixed for 90 seconds (1/10) (Kodafix; Eastman Kodak, Rochester, NY). The slides were thoroughly washed with distilled water and mounted in Gel mount (Biomeda, Foster City, CA). For double-labeling following in situ hybridization and for double immunohistochemistry, the alkaline phosphatase–anti–alkaline phosphatase method was performed using a commercially available kit (Dako, Hamburg, Germany). For all immunohistochemistry experiments, primary antibodies against macrophages (anti-CD68; Dako), fibroblasts (anti–prolyl-5-hydroxylase; Dako), activated lymphocytes (anti-CD45RO; Dako), and terminal complement complex (TCC) (anti-C5b–9; Dako) were used.

Western blotting. SF samples obtained from RA and OA patients were immediately frozen and stored at −70°C until used. Samples were diluted 1:1 in reducing SDS–polyacrylamide gel electrophoresis loading buffer (125 m*M* Tris, pH 6.8, 4% SDS, and 20% glycerol) and denatured by heating at 95°C for 5 minutes. Samples were electrophoresed in 12% SDS–polyacrylamide gels, and proteins were transferred to a nitrocellulose membrane.

For the detection of C3 and FB, blots were blocked for 1 hour at room temperature in phosphate buffered saline

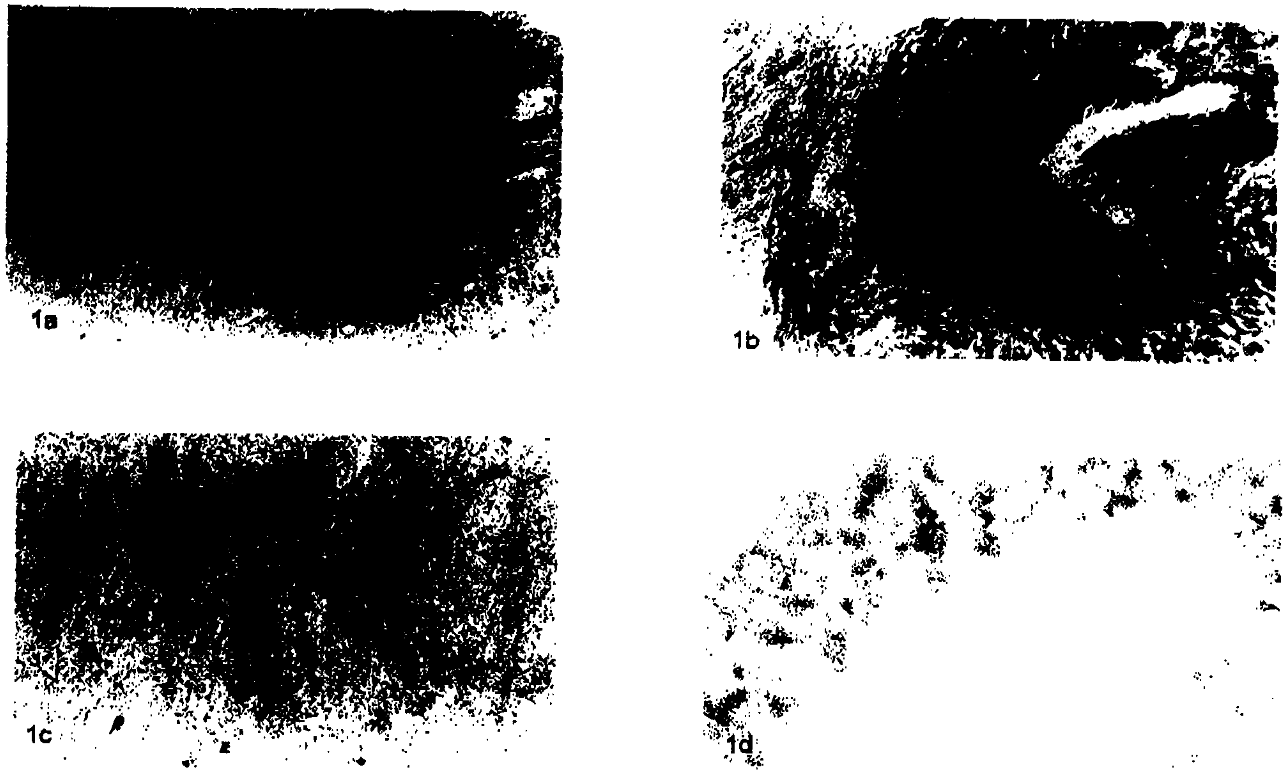

Figure 1. Complement proteins in synovial specimens. **a,** Intensive expression of complement protein C3 mRNA (**black staining**) around rheumatoid arthritis (RA) synovial microvasculature (**solid arrow**), and the lack of C3 expression in the lining layer (**open arrow**). In situ hybridization, antisense probe (RA06; original magnification × 400). **b,** High-power field of a serial section of the specimen shown in **a**. Note the intensive expression of complement protein C3 mRNA (**black staining**) near the vessels. In situ hybridization, antisense probe (original magnification × 800). **c,** Serial section of the specimen used in **a**. In addition to the C3 mRNA expression around the microvasculature, numerous fibroblast- and macrophage-like cells throughout the synovium express this complement protein. In situ hybridization (**black staining**), antisense probe (original magnification × 400). **d,** Limited expression of complement protein C3 mRNA (**black staining**) in osteoarthritis (OA) synovium. In situ hybridization, antisense probe (OA03; original magnification × 400).

(PBS; pH 7.5) containing 10% (weight/volume) nonfat dry milk, washed, and either polyclonal rabbit anti-C3 or FB antibodies (1.2 μg/ml; Quidel, San Diego, CA) in PBS containing 0.1% Tween 20 (volume/volume) and 1% nonfat dry milk (w/v) were added for 1 hour at room temperature. Blots were washed and then treated with a 1:5,000 dilution of a peroxidase-conjugated anti-rabbit IgG secondary antibody. The secondary antibody was detected using an enhanced chemiluminescence detection kit (Amersham Canada, Oakville, Ontario, Canada).

C3 and FB enzyme-linked immunosorbent assays (ELISAs). The levels of C3 and FB were determined using a previously described ELISA (27,28). The C3 assay detects C3 and C3 activation fragments including C3b and C3c, while the FB assay detects FB and the FB activation fragment Bb. All assays were run with a standard curve, nonspecific binding controls, and internal standards to ensure accuracy and reproducibility. All samples were run in duplicate, and the results are expressed as the mean of duplicate samples.

SCID mouse experiments. *Cell culture.* Culture of synovial fibroblasts was performed as described recently (29). Briefly, following enzymatic digestion, fibroblasts were grown in Dulbecco's modified Eagle's medium (Biochrom, Berlin, Germany) containing 10% heat-inactivated fetal calf serum (Gibco Life Technologies, Grand Island, NY), 100 units/ml penicillin, and streptomycin (PAA Systems, Linz, Austria), and cultured for 4 passages at 37°C in 10% CO_2. The synovial fibroblasts were stained for fibroblast markers by immunohistochemistry. More than 95% could be stained positively for the fibroblast enzyme prolyl 4-hydroxylase, and none was

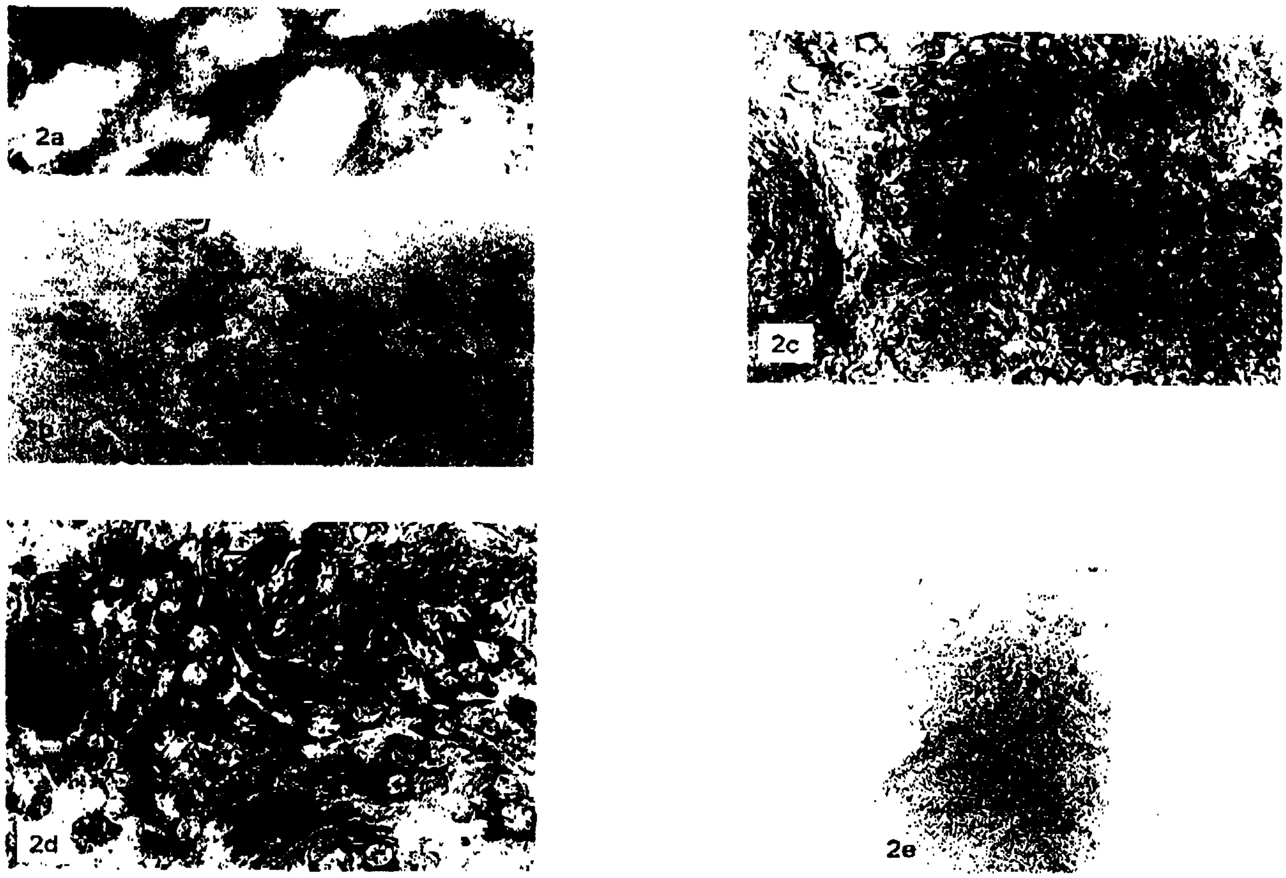

Figure 2. Expression patterns of mRNA for complement receptors C3aR and C5aR. **a,** C3aR mRNA in rheumatoid arthritis (RA) synovium (patient RA08). Expression of C3aR mRNA (**black staining**), showing a pattern different from that of C3, with the most intensive expression adjacent to small inflamed infiltrates. In situ hybridization, antisense probe (original magnification × 300). **b,** Control section using C3aR sense probe, illustrating the specificity of the probe by showing low background (original magnification × 300). **c,** Complement protein C5aR mRNA in RA synovium (patient RA09). Intensive expression of C5aR mRNA (**black staining**), showing a pattern different from that of C3 but similar to C3aR, with the most intensive expression in and around inflamed infiltrates. In situ hybridization, antisense probe (original magnification × 600). **d,** High-power field of a C5aR mRNA–positive area within RA synovium. Note the intensive expression of C5aR mRNA (**black staining**) in fibroblast-like cells (**arrows**) near the inflamed infiltrates. In situ hybridization, antisense probe (original magnification × 800). **e,** Lack of expression of complement protein C5aR mRNA (**black staining**) in osteoarthritis (OA) synovium (patient OA45). In situ hybridization, antisense probe (original magnification × 200).

positive for the macrophage marker CD68 or the neutrophil marker cathepsin G (results not shown). In addition, testing for mycoplasma contamination was performed routinely.

Transduction. The synovial fibroblasts were transduced with Ad5sTNFRp55:Ig as recently described (30). For adenoviral transduction in vitro, 4 fibroblast populations from patients with RA were grown to 80% confluence in 6-well plates (5×10^5 cells per well). The cells were washed and then transduced with 50 multiplicities of infection of Ad5sTNFRp55:Ig. Production of soluble tumor necrosis factor receptor (sTNFR) p55 was confirmed by ELISA (R&D Systems, Wiesbaden, Germany) prior to implantation (i.e., 48 hours after transduction).

Implantation. Four-week-old SCID mice (n = 12) were obtained from a germfree breeding colony (Charles River, Sulzfeld, Germany) and examined for macroscopic anomalies before and during surgery as well as for macropathologic and histopathologic abnormalities after being killed. On the day of implantation, normal human cartilage was obtained from nonarthritic knee joints of patients undergoing routine surgery at the University of Regensburg. Implantation of fibroblasts and cartilage was performed under sterile conditions using the

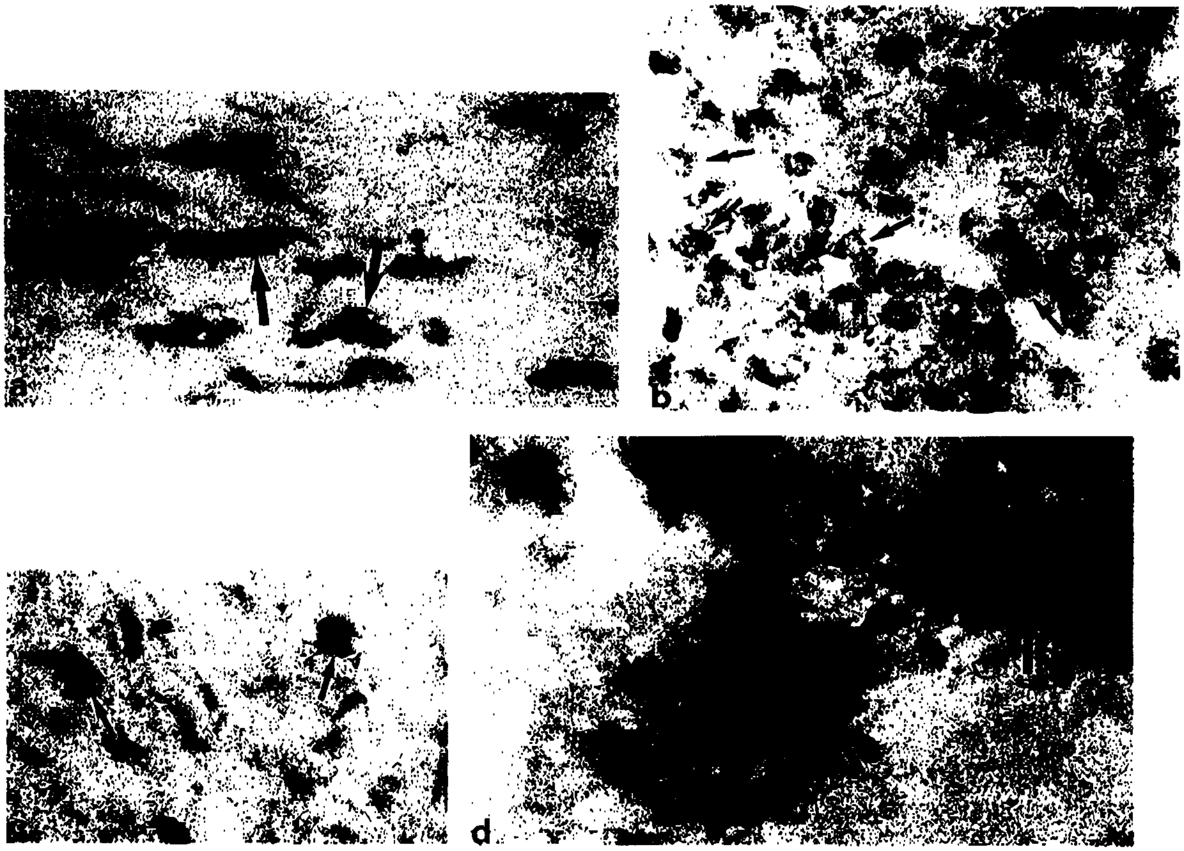

Figure 3. Expression patterns of mRNA for complement receptors C3aR and C5aR. a–d, High-power fields of C5aR and C3aR mRNA–positive areas within RA synovium. Double-labeling determined C5aR mRNA–expressing (**black staining**), 5-prolyl-hydroxylase+ (**red staining, arrows**) fibroblasts (**a**); C5aR mRNA–expressing (**black staining**) cells are found adjacent to CD68+ (**red staining, arrows**) macrophages (**b**). Conversely, C3aR mRNA (**black staining**) is expressed by numerous CD68+ macrophages (**red staining, arrows**) (**c**), and activated lymphocytes (**red staining, arrows**) (**d**). (Original magnification × 400.)

novel "inverse wrap" technique (30). Using 1 mouse for each implant, each experimental group (i.e., sTNFR p55–transduced synovial fibroblasts, LacZ-transduced synovial fibroblasts, and mock-transduced synovial fibroblasts) consisted of 4 mice, each implanted with synovial fibroblasts from 4 different patients. After 60 days, the mice were killed, and the implants were removed and embedded immediately in TissueTek embedding medium (Miles), snap-frozen, and stored at −70°C prior to histologic and immunohistochemical analysis for C3aR and C5aR production using the immunogold-silver method (see above).

RESULTS

Complement proteins could be detected in all RA synovial specimens examined. C3 mRNA was demonstrated most strongly around the synovial microvasculature (Figure 1a), showing the highest density closest to vessels (Figure 1b). In addition, C3 mRNA was synthesized by numerous fibroblast- and macrophage-like cells in the sublining (Figure 1c). In contrast, examination of C3 mRNA in a representative OA synovial specimen revealed only a few, single cells expressing this complement protein (Figure 1d).

Interestingly, mRNA for C3aR and C5aR showed an expression pattern quite different from that for C3 mRNA. C3aR was not detected in cells forming the terminal synovial vessels but was expressed by perivascular cells adjacent to small inflamed infiltrates (Figure 2a). Figure 2b shows a control section using the

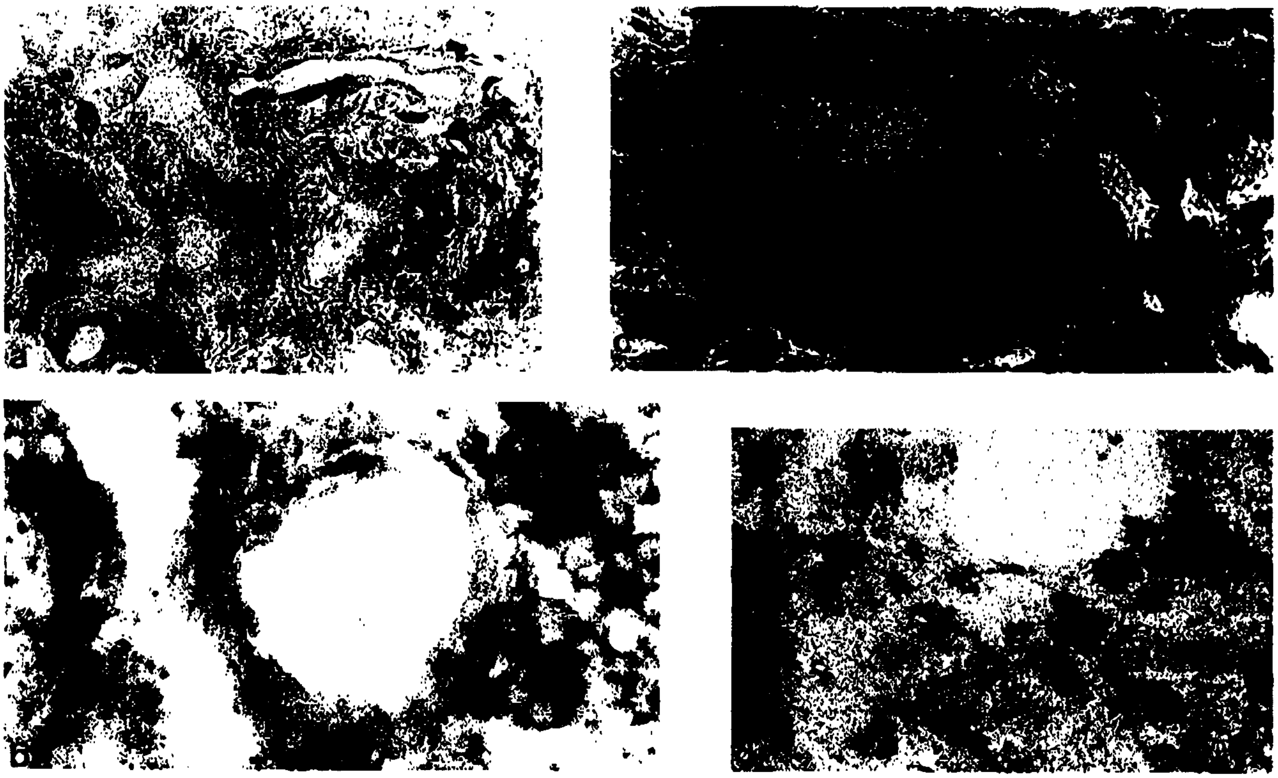

Figure 4. Expression patterns of factor B (FB). **a**, Intensive expression of complement protein FB mRNA in RA synovium (patient RA11). Note the pattern, which is similar to that of C5aR, and the clustered FB mRNA–expressing cells. In situ hybridization, antisense probe (original magnification × 600). **b**, High-power field of an FB mRNA–positive area within RA synovium (patient RA09). Note the intensive expression of FB mRNA (black staining) in macrophage/monocyte-like as well as some fibroblast-like cells. In situ hybridization, antisense probe (patient RA09, original magnification × 800). **c**, Terminal complement complex C5b–9 in RA synovium (patient RA09). Intensive expression of C5b–9 around the microvasculature, similar to that of C3. Immunohistochemistry (black staining) (original magnification × 800). **d**, High-power fields of a C5b–9-positive area within RA synovium. Double-labeling shows C5b–9 deposits (red staining, alkaline phosphatase–anti–alkaline phosphatase technique) near or surrounded by activated lymphocytes (black staining, immunogold-silver technique) (original magnification × 400). See Figure 2 for other definitions.

C3aR sense riboprobe and demonstrates low background staining. Similarly, microvasculature and perivascular cells showed very limited expression of C5aR mRNA. In contrast, cells near inflamed infiltrates, especially fibroblast-like cells adjacent to these infiltrates (Figure 2c), strongly expressed C5aR mRNA (Figures 2d and 3a). This pattern of expression was different from that of C3aR-expressing macrophages (Figure 3c) and lymphocytes (Figure 3d). In a representative inflamed OA synovial specimen, C5aR mRNA was nearly absent (Figure 2e).

The expression pattern of FB resembled that of C5aR, i.e., FB was absent in the cellular components of the microvasculature, but numerous clusters of FB-expressing cells were observed close to vessels (Figure 4a). The cellular phenotype was macrophage/monocyte-like; however, some cells expressing FB were also fibroblast-like (Figure 4b). This pattern of staining was similar to that observed for C3. The membrane attack complex, C5b–9, was readily detected around the microvasculature in RA synovium (Figure 4c), whereas in OA this TCC was absent. Double-labeling revealed that high amounts of C5b–9 were present predominantly close to or surrounded by activated lymphocytes (Figure 4d).

In addition to examining for changes in cellular expression of complement proteins, we analyzed SF for

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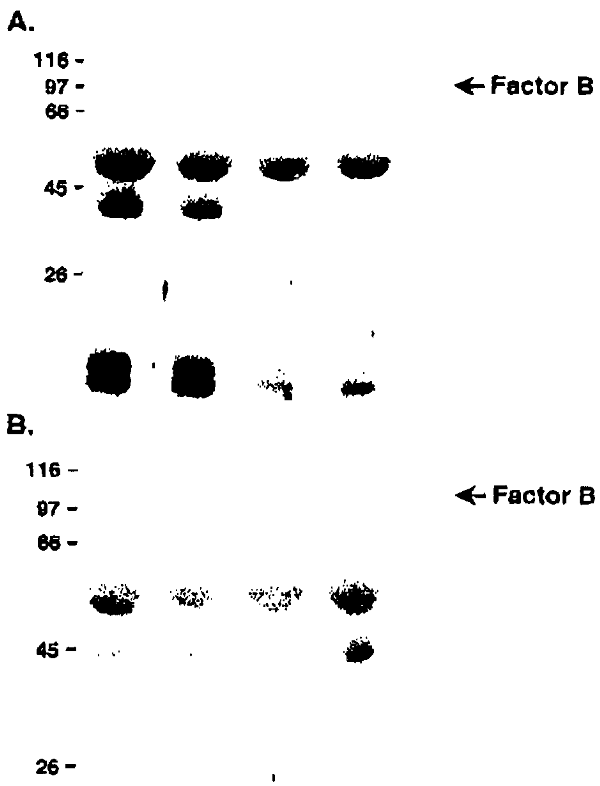

Figure 5. Factor B (FB) degradation fragments in rheumatoid arthritis (RA) and osteoarthritis (OA) synovial fluid (SF). A, Autoradiograph of Western blot probing for FB in SF of patients with RA. Shown are blots from representative individuals and the positions of molecular weight markers and purified FB. B, Same as A, except SF from patients with OA is shown.

changes in the levels of the complement activation components C3 and FB. For these studies, SF was analyzed by ELISA and Western blot. No difference in C3 protein concentration was seen between RA and OA samples, as determined by ELISA (average 82.6 versus 78.5 μg/ml, respectively). In addition, there was no apparent difference in the C3 banding pattern in RA versus OA SF (results not shown). In contrast, FB levels in RA samples were significantly lower than those in OA samples (average 28.3 versus 47.8 μg/ml), as determined by ELISA. Western blot analysis of SF showed a greater degradation of FB in samples from RA patients (Figure 5A) versus OA patients (Figure 5B). These data suggested that the lower FB levels in RA synovium may be due to greater proteolytic degradation.

Functional analysis of complement expression in the SCID mouse model for RA revealed that, similar to findings in the tissue experiments, RA synovial fibroblasts did not express C3aR. In addition, overexpression of sTNFR p55 did not stimulate C3aR synthesis in the transduced RA synovial fibroblasts (results not shown). In contrast, C5aR could be observed in a few, single fibroblasts around the coimplanted cartilage (Figure 6a), confirming the findings in ST mentioned above. This expression was not altered by adenovirus constructs encoding the marker gene LacZ (results not shown). Interestingly, following sTNFR p55 gene transfer, C5aR protein expression could be observed in numerous fibroblasts adjacent to the coimplanted cartilage (Figure 6b).

DISCUSSION

The findings presented in this report demonstrate for the first time the production of distinct complement components and complement factor mRNA in the inflamed RA synovium, particularly around small blood vessels. Specifically, we found increased expression of mRNA for C3 and FB, 2 key components involved in complement activation, in ST. The high expression of C3 and FB mRNA in situ supports the idea that they are produced locally in RA synovium and are not plasma derived, especially because C3 and FB were also present in OA SF, and FB concentrations were even lower in RA than in OA SF. Our data also confirm in situ expression of C3, as previously determined by Firestein et al (14). However, it is not clear whether the sublining lymphoid aggregates expressing C3 mRNA, as described by Firestein and colleagues (14), include macrophage-like cells similar to those we observed, or if they are truly lymphoid in nature. We would argue that the described lymphoid aggregates are probably macrophages, because T and B cells are not ready sources of C3 production.

The most unique finding of our study was the demonstration of increased expression of the receptors for the complement anaphylatoxins C3a and C5a. To date, no study has demonstrated whether local expression or changes in local expression of the receptors are significant, despite numerous studies quantifying C3 activation fragments and C5a levels in SF (10,11,13). Our findings would suggest that these complement activation fragments may serve not only to recruit infiltrating cells, but may play an important role in activating resident synovial cells as well. Additional studies are

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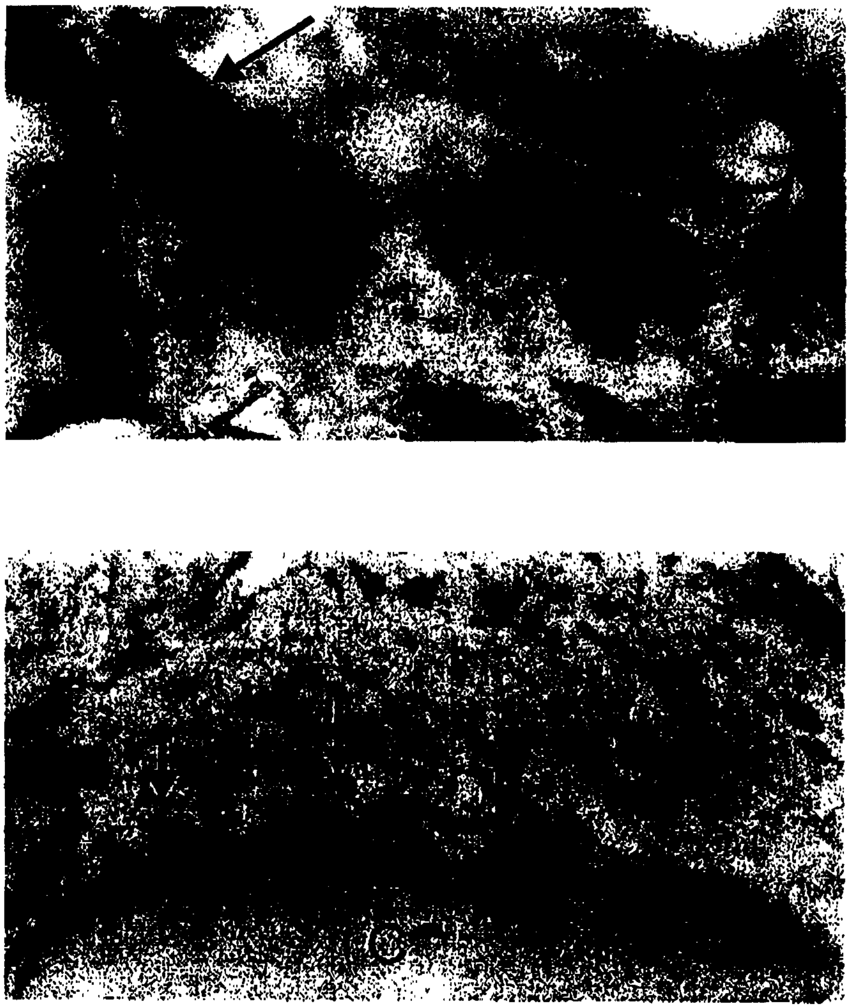

Figure 6. Complement expression in the SCID mouse model of RA. **a,** Immunohistochemical demonstration of C5aR in RA synovial fibroblasts. Note the limited expression of C5aR in only a few, single mock-transduced RA synovial fibroblasts (**arrows**) distant to the coimplanted normal human cartilage (C). **b,** Increase in C5aR expression in numerous soluble tumor necrosis factor receptor p55–transduced synovial fibroblasts (**arrows**) adjacent to the coimplanted normal human cartilage. See Figure 2 for definitions. (Original magnification × 400.)

required to support this hypothesis, but the presence of the receptor–ligand pair for both complement anaphylatoxin receptors makes a strong argument for the ability of local complement production to contribute to a vicious circle of inflammation.

Another novel finding of this study was that the expression pattern of each complement component (e.g., C3, FB, C3aR, C5aR, and C5b–9) was different in the RA synovial samples. For example, C3 mRNA was synthesized predominantly around terminal vessels, including the microvasculature itself, while C3aR was located on macrophages and activated lymphocytes between the microvasculature and inflamed infiltrates. In contrast, C5aR and FB mRNA were found predomi-

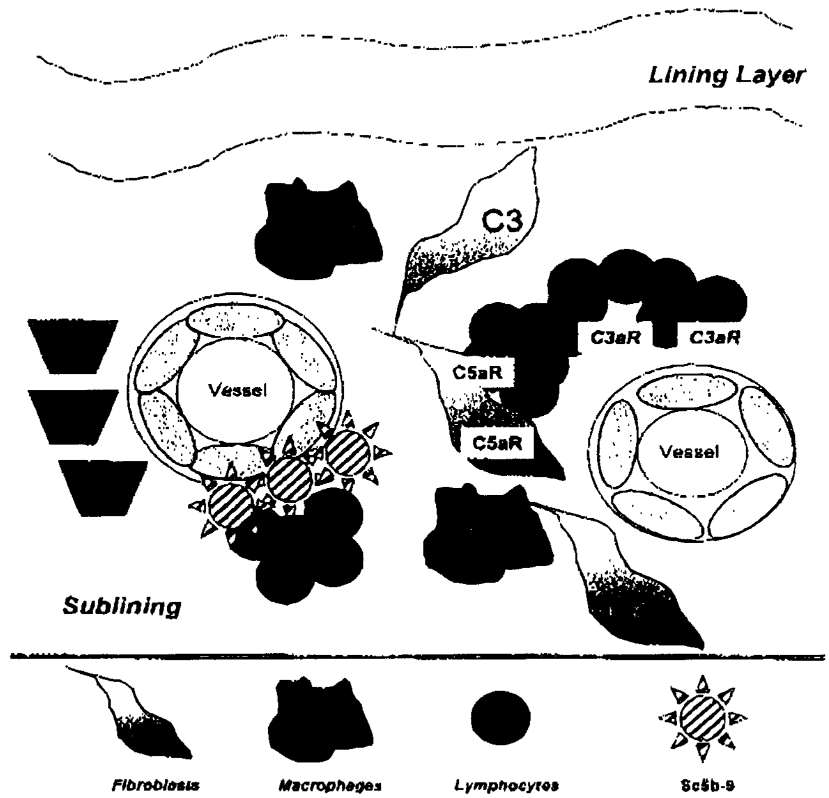

Figure 7. Schematic summary of study results. C3 mRNA is expressed most strongly around the synovial microvasculature synthesized by fibroblast- and macrophage-like cells in the sublining. In contrast, C3aR and C5aR show an expression pattern quite different from that of C3 mRNA. C3aR is expressed by perivascular cells adjacent to small inflamed infiltrates, whereas fibroblast-like cells strongly express C5aR mRNA. The expression pattern of factor B (FB) resembles that of C5aR, absent in the cellular components of the microvasculature, but numerous clusters of FB-expressing macrophage-like and fibroblast-like cells can be seen near vessels. The membrane attack complex, C5b–9, can also be readily detected around the microvasculature in RA synovium. Double-labeling reveals that high amounts of C5b–9 are present predominantly close to or surrounded by activated lymphocytes. See Figure 2 for other definitions.

nantly in and around inflamed infiltrates throughout the synovium. The TCC, however, showed a pattern similar to that of C3. The significance of this apparent differential distribution remains unclear, but it may reflect the level of tissue destruction and inflammation in a given patient sample, aside from other factors. Nonetheless, production and activation of complement appears to be a specific feature in RA rather than OA, since none of the complement components examined showed intense expression in OA synovium.

In addition to complement gene expression, we examined SF both from RA and OA patients for levels of C3 and FB by ELISA and Western blot analysis. Surprisingly, we found that C3 levels were essentially identical in SF from both groups. Given the inflamed nature of the RA synovium, we anticipated a lower level of C3 in this group due to degradation by numerous proteolytic enzymes. However, the comparable levels between the 2 groups may reflect an increased C3 synthesis in the RA synovium. The results of the in situ

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studies support this possibility. As assessed by Western blot, there were no significant differences in the C3 banding patterns between the samples from the RA and OA patients.

In contrast to the findings with C3, we observed that FB levels were ~50% lower in the SF of RA patients compared with that of OA patients. This, in part, is apparently due to increased degradation of FB in RA SF. However, we cannot rule out differences in FB synthesis in RA versus OA synovium, which may also account for the lower levels of FB we observed in RA synovium. The differential degradation of FB we observed between RA and OA SF samples was consistent for all samples examined and suggests that additional proteolytic enzymes besides factor D are involved in the degradation process. Long-term monitoring of changes in complement proteins in SF of RA and OA patients is required to determine potential mechanisms for the findings we report here.

With regard to human disease, the complement system has been shown to play a role in the pathogenesis of various immune-mediated disorders, including dermatologic, renal, neurologic, and rheumatic diseases (4–7). However, there is increasing evidence that the complement system could also play an important role in the pathogenesis of RA. Many studies have shown high levels of complement components and active complement metabolites C2, C3, C3a, C5a, and TCC C5b–9 in serum, SF, and ST (5–18). Furthermore, positive correlations have been shown between SF complement activation (e.g., levels of plasma C3dg, a C3 activation metabolite) and local as well as general disease activity in RA (10,31,32), and between C2 and C3 expression in RA synovium and inflammation (14). Complement activation could be induced by immune complexes consisting of type II collagen (CII) and CII autoantibodies (33) or by IgG and rheumatoid factor (34) in vitro.

Similar mechanisms are activated by the increased release of histamine in response to C5a by synovial mast cells, which have been shown to bear significant amounts of C5aR in RA compared with OA (35). Assembly of TCC on cell membranes results in the formation of the membrane attack complex (MAC) (4–6,36,37), which can lead to lytic attacks on synovial cells and chondrocytes, facilitated by low levels of the membrane-associated inhibitor of the MAC protectin (CD59) on synovial cells (19). Since synovial cells as well as other nucleated cells are rather resistant to lytic attacks (4–7), the primary effect of the MAC in RA appears to be sublytic attacks on the cells. This results in a release of reactive oxygen metabolites, prostaglandin E_2, leukotriene B_4, and interleukin-6 from synovial cells (4–7,36,37), which may lead to enhanced DNA damage in rheumatoid synovium (38) and increased collagenase-specific mRNA expression by synovial fibroblasts (39). These effects are supported by low concentrations of the fluid-phase MAC inhibitors clusterin and vitronectin in SF (18). Of interest, the "hot zone," in which complement activation results in actual damage, appears to be the microvasculature, a fact that is illustrated by the intensive amounts of C5b–9 close to activated lymphocytes in this area.

Certain complement proteins may also contribute to joint destruction. C1s, which is increased by TNFα in chondrocytes, is involved in cartilage degradation (40). More important, complement, in particular C1s in conjunction with matrix metalloproteinase 9, has a physiologic role in remodeling of cartilage in the growth plate and in fracture healing (41–43).

Findings in studies using animal models of RA also support the hypothesis that complement is required for the development of arthritis. Wang et al (44) observed that in DBA/1LacJ mice with collagen-induced arthritis (CIA), systemic administration of a specific anti-C5 monoclonal antibody inhibits terminal complement activation in vivo and prevents the subsequent onset of arthritis in immunized mice, and ameliorates established disease. Wang and colleagues also concluded that activated TCC plays an important role in the induction as well as the progression and manifestation of disease. Moreover, C5-deficient mice do not develop CIA (45–48). Similarly, Goodfellow et al (49) showed that in a rat model of antigen-induced arthritis, intraarticular injection of soluble complement receptor 1 prevented the onset of and ameliorated established disease.

Our data reveal that in the SCID mouse model for RA, which is currently used to evaluate the effects of various inhibitory molecules on cartilage destruction in vivo (29), C5aR expression is up-regulated by sTNFR p55 overexpression, indicating that TNF-dependent pathways in rheumatoid synovium may be linked directly to components of the complement cascade. We have previously observed that TNFα induces the expression of C5aR on neurons in a murine model of bacterial meningitis (24). Moreover, the demonstration of C5aR-expressing synovial fibroblast–like cells similar to the immunohistochemical findings in RA synovium supports the hypothesis that "invading" RA synovial fibroblasts may be phenotypically different from "inflamed" RA synovial fibroblasts.

In summary, we conclude that complement factors and complement receptors play an important role in

RA, and that synthesis and activation of complement takes place at distinct sites within rheumatoid synovium, as summarized in Figure 7. Specific modulation and inhibition of local complement production could therefore be an attractive target for RA therapy, as illustrated by recent data derived from a C5-deficient mouse model (48) as well as by an ongoing phase II clinical trial examining the use of anti-C5 antibodies in RA (50).

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